Int. a1

1,5-Benzodiazepine Derivatives

This invention relates to novel 1,5-benzodiazepine derivatives, to processes for their preparation, to pharmaceutical compositions containing them and to their use in medicine. More particularly, it relates to compounds which exhibit agonist activity for CCK-A receptors.

Cholecystokinin (CCK) is a peptide found in the gastrointestinal tract and the central nervous system. see A.J. Prange *et al.*, *Ann. Reports Med. Chem. 17,* 31, 33 (1982), J. A. Williams, *Biomed Res. 3*, 107 (1982) and V. Mutt, *Gastrointestinal Hormones*, G.B.J. Green, Ed., Raven Press, N.Y., 169. CCK has been implicated *inter alia* as a physiological satiety hormone involved in appetite regulation, see Della-Ferra *et al*, *Science, 206*, 471 (1979), Saito *et al.*, *Nature, 289*, 599, (1981), G.P. Smith, *Eating and Its Disorders*, A.J. Stunkard and E. Stellar, Eds, Raven Press, New York, 67 (1984), as a regulator of gallbladder contraction and pancreatic enzyme secretion, an inhibitor of gastric emptying, and as a neurotransmitter, see A.J. Prange, *supra*, J.A. Williams, *Biomed Res., 3*, 107 (1982), J.E. Morley, *Life Sci. 30*, 479, (1982). Gastrin is a peptide involved in gastric acid and pepsin secretion in the stomach, see L. Sandvik, *et al., American J. Physiology, 260*, G925 (1991), C.W. Lin, *et al., American J. Physiology, 262*, G1113, (1992). CCK and gastrin share structural homology in their C-terminal tetrapeptide: Trp-Met-Asp-Phe.

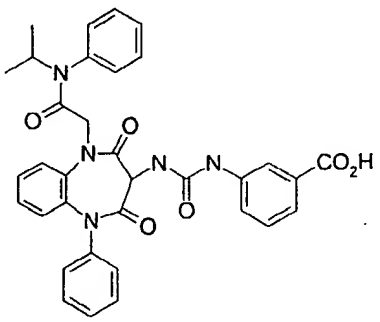
Two subtypes of CCK receptors have been identified, designated as CCK-A and CCK-B, and both have been found in the periphery and central nervous systems. It has recently been reported that CCK-B receptors are similar to the gastrin receptor, see Pisegna, J.R., de Weerth, A, Huppi, K, Wank, S.A., *Biochem. Biophys. Res. Commun. 189*, 296-303 (1992). CCK-A receptors are located predominantly in peripheral tissues including the pancreas, gallbladder, ileum, pyloric sphincter and vagal afferent nerve fibers; CCK-A receptors are found to a lesser extent in the brain, see T.H. Moran, *et al., Brain Res., 362,* 175-179

(1986), D.R. Hill, *et al., Brain Res, 4545*, 101, (1988), D.R. Hill, *et al., Neurosci Lett., 89*, 133, (1988), R.W. Barret, *et al., Mol. Pharmacol., 36*, 285, (1989), D.R. Hill, *et al., J. Neurosci, 10,* 1070 (1990), V. Dauge *et al., Pharmacol Biochem Behac., 33,* 637, (1989), while CCK-B receptors are found predominantly in the brain, see V.J. Lotti and R.S.L Chang, *Proc. Natl. Acad. Sci. U.S.A., 83,* 4923 (1986), J.N. Crawley, *Trends Pharm. Sci., 88*, 232, (1991).

CCK agonist activity has been linked to inhibition of food intake in animals and thus weight loss, see Della-Fera, *et al, supra,* K.E. Asin, *et al, Intl. Conference on Obesity*, abstract pp.40 (1990). It has been suggested that CCK acts in the periphery through vagal fibers and not directly on the brain to produce satiety, see Smith, G.P. and Cushin, B.J., *Neuroscience Abstr., 4*, 180 (1978), Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., and Simansky, K.J., *Science, 212,* 687-689, (1981).

U.S. Patent No. 5,646,140 (Sugg, et al.) discloses certain 3-amino 1,5-benzodiazepine compounds which exhibit agonist activity for the CCK-A receptor thereby enabling them to modulate the hormones gastrin and cholecystokinin (CCK) in mammals. See in particular, the compound of Example 7. Certain of these compounds also exhibit antagonist activity at CCK-B receptors.

Briefly, in one aspect, the present invention provides an enantiomerically enriched compound of Formula (I) or a pharmaceutically acceptable salt or solvate thereof.



(I)

The compound of Formula (I) is 3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid. This compound has a chiral carbon on the benzodiazepine ring. Applicants have found that the enantiomer which rotates light in the positive direction, under the conditions described below, is preferred. This enantiomer which is hereinafter referred to as the (+) enantiomer has the (S) configuration according to the Cahn Ingold Prelog convention. Applicants have found that this isomer has improved properties over the racemic mixture and is therefore more suitable than the racemic mixture for the treatment of obesity and other CCK-A mediated diseases or conditions. As used herein, "enantiomerically enriched" means that there is more of the (+) enantiomer than the (-) enantiomer as opposed to the racemic mixture which has equal amounts of each isomer. As used herein " the compound of this invention" or "the enantiomerically enriched compound of this invention", and expressions containing these or similar phrases, include pharmaceutically acceptable salts and solvates thereof. The "(+) enantiomer" refers to the optical rotation of the enantiomer and not to salts and solvates thereof. Preferred salts and solvates will be salts and solvates of the (+) enantiomer of the compound of Formula (I) regardless of the optical rotation of the salt or solvate.

Preferably, the (+) enantiomer is at least %90 of the total amount of the enantiomerically enriched compound. More preferably, the (+) enantiomer is at least %96 of the total amount of the compound. Most preferably, the (+) enantiomer is at least %99 of the total amount of the compound.

The (+) enantiomer of the present invention exhibits CCK-A agonist activity and can be considered a full cholecystokinin agonist in that it binds to CCK-A receptors and fully stimulates gallbladder contraction and reduces feeding in animal paradigms. For example, (+) enantiomer of this invention should be useful for the treatment of obesity as well as related pathologies, such as hypertension,

gallbladder stasis, and diabetes, indirectly through weight loss and directly through CCK-A mediated delayed gastric emptying. Moreover, the (+) enantiomer disclosed herein provides for new approaches for inducing satiety, providing for appetite regulation and modifying food intake in mammals, especially humans, to regulate appetite, treat obesity and maintain weight loss.

Therefore, in a further aspect of the present invention, there is provided herein a method for the treatment, in a mammal, including man, of a CCK-A mediated disease or condition comprising administering to the patient a therapeutically effective amount of the (+) enantiomer of this invention.

According to another aspect, the present invention provides the use of the enantiomerically enriched compound of this invention or a pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for the treatment of CCK-A medicated diseases or conditions.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established diseases or symptoms. Moreover, it will be appreciated that the amount of the preferred enantiomer of the invention required for use in treatment will vary with the nature of the condition being treated and the age and the condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian. In general, however, doses employed for adult human treatment will typically be in the range of 0.02 - 5000 mg per day, e.g., 1-1500 mg per day. The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

While it is possible that the enantiomerically enriched compound of the present invention may be therapeutically administered as the raw chemical, it is

preferable to present the active ingredient as a pharmaceutical composition. Accordingly, the present invention further provides for a pharmaceutical composition comprising the enantiomerically enriched compound of this invention together with one or more pharmaceutically acceptable carriers and/or excipients therefore and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) and/or excipients therefor must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations of the present invention include those especially formulated for oral, buccal, parenteral, implant, topical or rectal administration, however, oral administration is preferred. For buccal administration, the composition may take the form of tablets or lozenges formulated in conventional manner. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, (for example, syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinylpyrrolidone), fillers (for example, lactose, sugar, microcrystalline cellulose, maize-starch, calcium phosphate or sorbitol), lubricants (for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica), disintegrants (for example, potato starch or sodium starch glycollate) or wetting agents, such as sodium lauryl sulphate. The tablets may be coated according to methods well-known in the art, including enteric coatings.

Alternatively, the preferred enantiomer of the present invention may be incorporated into oral liquid preparations such as aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, for example. Moreover, formulations containing these the preferred enantiomer may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents such as sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel or hydrogenated edible fats; emulsifying agents such as lecithin, sorbitan mono-

oleate or acacia; non-aqueous vehicles (which may include edible oils) such as almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; and preservatives such as methyl or propyl p-hydroxybenzoates or sorbic acid. Such preparations may also be formulated as suppositories, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

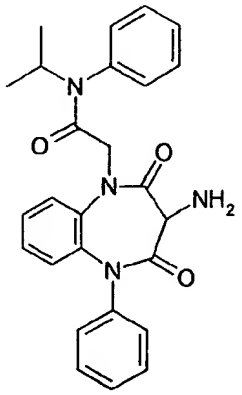
Additionally, compositions the present invention may be formulated for parenteral administration by injection or continuous infusion. Formulations for injection may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile, pyrogen-free water) before use.

The composition according to the invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Accordingly, the preferred enantiomer of the invention may be formulated with suitable polymeric or hydrophobic materials (as an emulsion in an acceptable oil, for example), ion exchange resins or as sparingly soluble derivatives as a sparingly soluble salt, for example.

The compositions according to the invention may contain between 0.1 - 99% of the active ingredient, conveniently from 30 - 95% for tablets and capsules, and 3 - 50% for liquid preparations.

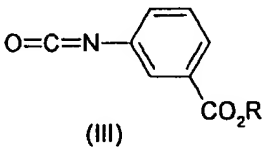
The (+) enantiomer of this invention can made by first making the racemic mixture as described in Example **7** in U.S. Patent No. 5,646,140 and then separating the enantiomers by chiral chromatography.

Alternatively the (+) enantiomer may be prepared by reaction of the appropriate enantiomer, namely the (S)-enantiomer of the amine of formula (II);

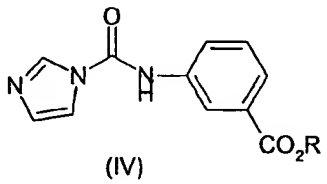


(II)

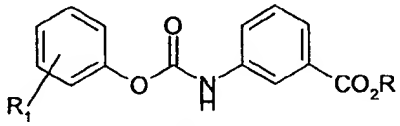
with an isocyanate of formula (III; wherein R is a carboxyl protecting group e.g. t-butyl), an imidazolide of formula (IV; wherein R is a carboxyl protecting group e.g. t-butyl) or an optionally substituted phenyl carbonate of formula (V; wherein R is a carboxyl protecting group e.g. t-butyl and R_1 is hydrogen or a conventional phenyl substituent e.g. NO_2) followed by removal of the carboxyl protecting group R.



(III)



(IV)



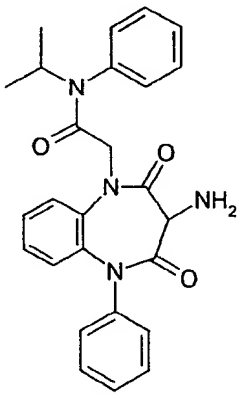
(V)

The reaction conveniently takes place in the presence of a suitable solvent such as an ether (e.g. tetrahydrofuran) or a halohydrocarbon (e.g. dichloromethane) or nitrile (e.g. acetonitrile) at a temperature in the range of 0-80°C.

Conveniently the required enantiomer of the amine (II) may be used in the form of a salt thereof e.g. R-camphorsulphonic acid salt and in this embodiment the reaction may be carried out in the presence of a base e.g. of a tertiary amine such as triethylamine.

The hydrolysis of the carboxyl protecting group may be carried out using conventional procedures. (Protecting groups in Organic Synthesis T. Greene, Ed, Wiley Interscience, New York, p168, 1981). Thus for example when R is a t-butyl group this may be removed by hydrolysis with an appropriate acid such as hydrochloric acid, trifluoroacetic acid or formic acid using established procedures. For example by reaction with hydrochloric acid in a solvent such as 1,4-dioxane; or by reaction with formic acid in a solvent such as acetone or aqueous acetone and with heating.

The required (S)-enantiomer of the amine of Formula (II);

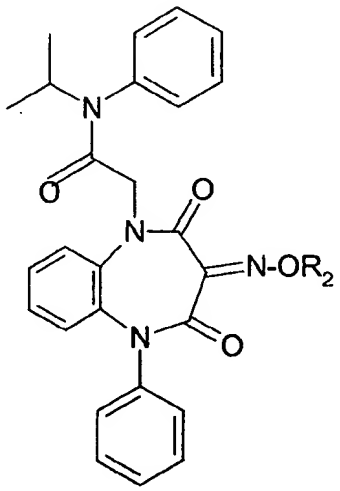


(II)

can be prepared by resolution of the corresponding racemic amine via chiral HPLC chromatography or through crystallization-induced asymmetric resolution via the R-camphorsulfonic acid salt.

The racemic amine (II) can be prepared by the method described in Intermediate 11 of US Patent No. 5,646,140.

Alternatively, the racemic amine (II) may be prepared by concomitant reduction and hydrogenolysis of the oxime (VI) wherein R_2 is an optionally substituted benzyl group.

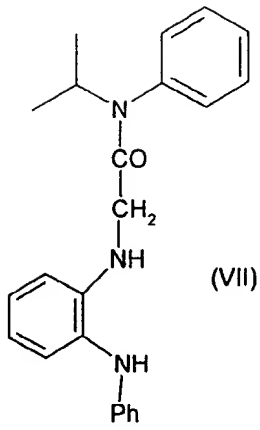


(VI)

The reaction is conveniently carried out using a suitable palladium catalyst e.g. palladium on carbon e.g. 5% Pd/on charcoal in the presence of hydrogen or aqueous ammonium formate and in a solvent such as an aqueous alkanol e.g. ethanol, isopropanol or industrial methylated spirits, or tetrahydrofuran. Conveniently the reaction is carried out with heating e.g. 40-80° such as 60°C.

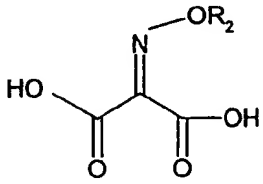
Examples of suitable R_2 groups for use in the reaction include benzyl, or substituted benzyl, such as p-methoxybenzyl, or benzhydryl.

The oxime (VI) may be prepared by reaction of the ortho-phenylene diamine derivative (VII)



(VII)

with an activated derivative of the di acid (VIII) wherein R_2 is an optionally substituted benzyl group.



(VIII)

Conveniently the activated derivative of the di acid (VIII) is the corresponding diacyl halide e.g. chloride and this is prepared in situ by reaction of the di acid (VIII) with an oxalyl halide e.g. oxalyl chloride. The reaction is conveniently carried out on an aprotic solvent such as an ester e.g. ethyl acetate, toluene, dichloromethane, dimethoxyether or mixtures thereof and in the presence of dimethylformamide.

The di acid (VIII) is conveniently prepared by reaction of a di alkylketomalonate e.g. diethyl ketomalonate with the corresponding hydroxylamine R_2ONH_2 in a solvent such as an alkanol e.g. ethanol or industrial methylated spirits and in the presence of a base e.g. pyridine, followed by hydrolysis of the corresponding di alkyl-oximino malonate using aqueous sodium hydroxide.

In a further aspect the invention provides a process for preparing the (S) enantiomer of the compound of formula (I) substantially free of the (R)

enantiomer thereof from the racemic amine (II) as described above wherein the racemic amine (II) has been prepared from the oxime (VI) as described above and more particularly, wherein the oxime (VI) has been prepared from the compounds (VII) and (VIII).

Isocyanates of Formula (III) may be purchased or prepared by the reaction of the corresponding amine (VI) with phosgene or triphosgene in a suitable solvent such as methylene chloride. Imidazolides of Formula (IV) can be prepared by treatment of the corresponding amine (VI) with carbonyl diimidazole in a suitable solvent (dichloromethane, ether, tetrahydrofuran) at a temperature ranging from 0 - 80° C (conveniently at room temperature). The optionally substituted phenyl carbamates of Formula (V) can be prepared by the reaction of the corresponding amine (VI) with the optionally substituted phenyl chloroformate in the presence of a base (pyridine, triethylamine) in a suitable solvent (dichloromethane) and at a temperature of 0 - 50° C. The amines of formula (VI) are either known compounds and can be prepared by procedures analogous to those used to prepare the known compounds.

The following examples, which are non-limiting, illustrate the invention.

In the Examples the abbreviations EtOAc = ethyl acetate; MeOH = methanol, DMF = N, N-dimethylformamide; IPA = isopropyl alcohol; IMS = industrial methylated spirits.

Intermediate 1

(+)-2-(3-Amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo-[b][1,4]diazepin-1-yl)-N-isopropyl-N-phenylacetamide camphorsulfonic acid salt

(+/-)-2-(3-Amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo-[b][1,4]diazepin-1-yl)-N-isopropyl-N-phenylacetamide (10g) and R-camphorsulfonic acid (4.98g) were stirred in tetrahydrofuran (35ml) and toluene (65ml) to give a solution. The

solution was heated to 70°C with formation of a suspension. Water (0.4ml) was added followed by a solution of 2-pyridinecarboxaldehyde (0.24g) in toluene (5ml). The mixture was heated at 70°C for 3h and then cooled to 25°C over 5h and stirred at 25°C for 16h. The suspension was chilled to 0-5C for 1.5h. The solid was collected by filtration washing with toluene/tetrahydrofuran (2:1) (10ml). Drying in vacuo at 50°C yielded the title compound as a white solid (12.6g). Chromatographic analysis: Eluent: 30%Isopropyl Alcohol, 70% Heptane + 0.05% Diethylamine; Column: 25cm x4.6mm i.d., Chiralpak AD; Flow rate: 1ml/minute; Temperature: 40 degrees C; Detection: UV 230nm; Injection volume: 10 µL; Sample solution: 0.1mg/ml; sample in 30% Isopropyl alcohol, 70% Heptane. Sample solutions were injected immediately after preparation. Retention times: (+) enantiomer, 8.2 minutes. The unwanted (-) Enantiomer (12.7minutes) was below limits of detection.

Intermediate 2

3-Nitrobenzoic acid t-butyl ester

Potassium t-butoxide (3.82g) was added to a solution of 3 nitrobenzoyl chloride (5.00g) in anhydrous tetrahydrofuran (70 ml) and stirred under nitrogen for 2 hrs. The reaction mixture was concentrated *in vacuo* and partitioned between dichloromethane and water. After separating the phases, the aqueous layer is back-extracted with ethyl acetate. The organic layers were combined, dried over anhydrous magnesium sulfate, filtered and then concentrated *in vacuo*. The crude product was purified on flash grade silica gel using 0-5% gradient of ethyl acetate in n-hexane. Fractions containing the product were combined, concentrated *in vacuo*, and then dried under high vacuum to provide the title compound as an oil (3.82g). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.63 (s, 9H); 7.62 (t, J=7.9 Hz, 1H); 8.29-8.41 (m, 2H); 8.78-8.80 (m, 1H). MS (CI): $[M+H]^+$ = 224.

Intermediate 3

3-Amino-benzoic acid t-butyl ester

A solution of 3-nitro-benzoic acid t-butyl ester (3.77g), in absolute ethanol (50 ml) was combined with palladium on carbon (10 wt%, 0.30 g) and stirred under atmospheric hydrogen for approximately 3 hrs. The reaction mixture was filtered through a pad of diatomaceous earth and then concentrated *in vacuo* to an oil which crystallized when dried under high vacuum providing the title compound as a tan solid (3.28g). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.58 (s, 9H); 6.79-6.87 (m, 1H), 7.19 (t, J=8.5 Hz, 1H); 7.24-7.34 (m, 1H); 7.38 (d, J=8.0 Hz, 1H). MS (CI): $[M+H]^+$ = 194.

Intermediate 4

3-Isocyano-benzoic acid t-butyl ester

Triphosgene (13.428g) was added to a solution of 3-amino-benzoic acid t-butyl ester (26.50g) and triethylamine (38.23ml) in anhydrous tetrahydrofuran (600 ml) at 0-5 °C. The reaction mixture was stirred at 0-5 °C for 2h, then concentrated *in vacuo* to a white solid. The crude product was slurried in hexane (500 ml), filtered, and the filtrate was concentrated *in vacuo* to afford the title compound as an oil (21.54 g, 71.6%). The crude isocyanate was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ= 1.59 (s, 9H); 7.23 (bd, J=7.8 Hz, 1H); 7.36 (t, J=7.8 Hz, 1H); 7.69 (bs, 1H); 7.81 (d, J=7.8 Hz, 1H).

Intermediate 5

(+)3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid t-butyl ester

Method A

Intermediate (I; 66.30g) was slowly added to a solution of 3-Isocyano-benzoic acid t-butyl ester (21.54g) in anhydrous tetrahydrofuran (750 ml). Triethylamine (13.70ml) was added dropwise to the reaction mixture. The resulting reaction mixture was stirred at ambient temperature overnight. The reaction mixture was poured into water (3000 ml) to afford a white solid. The solid was collected by filtration washing with water (3 X 500 ml). Drying by vacuum filtration yielded the

title compound as a white solid (65.01g). The crude title compound was used without further purification. Chromatographic characterization: Eluent: 30%Isopropyl Alcohol, 70% Heptane + 0.05% Diethylamine. Column: 25cm x4.6mm i.d., Chiralpak AD; Flow rate: 1ml/minute; Temperature: 40 degrees C; Detection: UV 230nm; Injection volume: 10µL; Sample solution: 0.1mg/ml sample in 30% Isopropyl alcohol, 70% Heptane. Sample solutions were injected immediately after preparation. Retention times: (+) enantiomer: 15.6 minutes. The unwanted (-) enantiomer: (13.3 minutes) was below the limits of detection.

Method B

To a suspension of carbonyl diimidazole (13.2g) in dichloromethane (55ml) stirring at 20°C was added dropwise a solution of 3-Amino-benzoic acid t-butyl ester (15.8g) in dichloromethane (40ml) over 30 mins. The resulting solution was stirred at 20°C for 1 hour. To this solution was added a solution of Intermediate 1 (50g) in dichloromethane (130ml) over 5 minutes. The reaction was quenched by addition of water (200ml) and stirred for 10 minutes. The phases were separated and the organic phase washed with water. The organic phase was concentrated at atmospheric pressure and 100ml dichloromethane was removed by distillation. *t*-Butyl methyl ether (700ml) was added and the mixture was stirred overnight at 20°C. The solid was collected by filtration and washed with *t*-Butyl methyl ether (100ml) and dried *in vacuo* at 45°C to provide the title compound as a white solid (42g, 63mmol).

Intermediate 6

Diethyl 2-[(benzyloxy)imino]malonate

Di-ethylketomalonate (60g) was added at 20°C to a stirred suspension of O-benzylhydroxylamine (57.8g) in IMS (500ml) containing pyridine (30ml). The reaction was heated at 75°C for 4hr. The reaction was cooled and solvents removed under reduced pressure. The residue was partitioned between EtOAc (500ml) and water (300ml) and the organic layer separated, washed with water

(250ml) and dried over $MgSO_4$. Solvents were evaporated to give the title compound 95.3g, as a colourless oil (99%th, ca 3%w/w residual EtOAc) which was used without further purification.

1H NMR (300MHz, $CDCl_3$) 7.4 (m, 5H), 5.35 (s, 2H), 4.35 (m, 4H), 1.3 (m, 6H).

Intermediate 7

2-[(benzyloxy)imino]malonic acid

To a solution of Intermediate 6 (40g) in MeOH (80ml) was added 2M NaOH (200ml) over 20 mins. The reaction was stirred at room temperature for 2hr. MeOH was removed under reduced pressure and the residual solution was acidified to pH 2 by dropwise addition of conc.HCl (~30ml) while cooling to maintain the temperature below 35°C. A thick white slurry was formed which was diluted with water (50ml) to aid mobility. The solids were collected by filtration, washed with chilled water (25ml) and dried in vacuo at 55°C to give the title compound as a white solid (17g) found to contain ca.10%w/w residual inorganic salts. Corrected yield ~ 45%th. Used without further purification.

1H NMR (300MHz, D_2O) 7.4 (m, 5H), 5.2 (s, 2H)

Intermediate 8

2-[-3-[(Benzyloxy)imino]-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-1,5-benzodiazepinyl)-N-isopropyl-N-phenylacetamide

Oxalyl chloride (38.3g) was added dropwise (~1hr) to a stirred suspension of Intermediate 7 (40g, corrected for salt content to 31.4g) in EtOAc (200ml) containing DMF (0.5ml, 5 mol%). The mixture was stirred at 25°C for 0.5 hour then filtered through a pad of Dicalite, washing with EtOAc (40ml) to give a clear yellow solution. The solution was added (~5mins) to a stirred slurry of N-isopropyl-N-phenyl-2-(2-phenylaminophenylamino)-acetamide (50g) in EtOAc (120ml) at 25°C. The mixture was warmed to 60°C and a dark purple solution formed. After 1hr, EtOAc (200ml) was removed by atmospheric distillation. IPA

(120ml) and water (40ml) were added and the mixture distilled further to remove more solvent (80ml). IPA (40ml) and water (40ml) were added and a further amount of solvent was distilled out (80ml). The reaction mixture was cooled to 25 °C over 1.5hr and the solids collected by filtration. The solids were washed with IPA (2 x 120 ml), water (1 x 120ml) and finally IPA again (1 x 40ml) then dried in vacuo at 55°C to give the title compound as a salmon pink powder (56.6g).

1H NMR (300MHz, $CDCl_3$) 2:1 mixture of isomers about the oxime 7.6-6.95 (m. 18H), 6.9 (t 1H), 5.3 (m, 2H), 4.95 (m, H), 4.65 (d, 0.33H), 4.4 (d, 0.67H), 4.1 (d, 0.67H), 4.0 (d, 0.33H), 0.95 (m, 6H)

Intermediate 9

(±)-2-(3-Amino-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydrobenzo-[b][1,4]diazepen-1-yl)-N-isopropyl-N-phenylacetamide

To a stirred suspension of Intermediate 8 (3g) and ammonium formate (2.08g) in IMS (30ml) and water (3ml) was added 5% Pd/C (50% w/w water) (0.25g). The mixture was heated under a nitrogen atmosphere at 60°C overnight. The hot reaction mixture was filtered through Dicalite to remove the catalyst. The catalyst was washed with hot IMS (60ml) and filtered. The filtrates were concentrated under reduced pressure to give the title compound as a white solid (2.34g).

Example 1

Chromatographic Resolution of Enantiomers

(+) and (-)-3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid.

Racemic 3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid was prepared as described for example 7 in U.S. Patent No. 5,646,140 and resolved by chiral HPLC under the following conditions: a 250 x 4.0 µm (id) column, 5um Diacel Chiracel OD-R; the eluent was 80:20:0.1:1, 80 parts acetonitrile, 20 parts water, 0.1 part triethylamine, and 1 part acetic acid; the UV detection wavelength was

230nm; the temperature was ambient; the flow rate was 1ml/min; and the injection volume was 20ul. Under these conditions the (+) enantiomer had a retention time of 6.50 minutes and the (-) isomer had a retention time of 3.89 minutes.

Example 2

(+)-3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid

Method A

(+)-3-{3-[1-(Isopropyl-phenyl-carbamoylmethyl)-2,4-dioxo-5-phenyl-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-3-yl]-ureido} benzoic acid t-butyl ester (Intermediate 5: 65.01g) was stirred in 4N hydrochloric acid in dioxane (280 ml) at ambient temperature for 6h. The reaction mixture was concentrated *in vacuo* and the resulting solid/oil was triturated in water to afford a white solid. The white solid was collected by filtration and washed with water (2 X 500 ml). The crude product was dissolved in hot acetone (250 ml) and water (275 ml) was added until the solution became cloudy. Additional acetone (40 ml) was added and the solution was heated until a clear solution was obtained. The solution was set aside and allowed to cool. The resulting white solid was collected by filtration washed with water (3 X 100 ml) and dried under house vacuum (20-25 in Hg) at 40-50 °C to provide the title compound as a white solid (40.496g). Analyzed for purity by chiral chromatography (see chromatographic resolution protocol). Optical rotation (0.712g in 100mL acetone) $[\alpha]_D$ = +84.3. ^{1}H NMR (300 MHz, DMSO) δ= 0.95 (d, J=7.5 Hz, 3H); 0.97 (d, J=7.2 Hz, 3H); 4.18 (d, J=16.7 Hz, 1H); 4.48 (d, J=16.7 Hz, 1H); 4.78 (m, 1H); 5.02 (d, J=7.8 Hz, 1H); 6.91 (d, J=7.8 Hz, 1H); 6.95 (bd, J=8.1 Hz, 1H); 7.22-7.57 (m, 19H); 8.00 (s, 1H); 9.34 (s, 1H); 12.78 (s, 1H). MS (ES): [M+1] = 606.1; [M+Na] = 628.1; [M-1] = 604.1.

Method B

Intermediate 5 (5g,) was added to a mixture of acetone (15ml) and formic acid (25ml) at room temperature and the resulting suspension was heated to 55°C

and stirred for 5h. Water (30ml) was added dropwise to the solution ensuring the contents temperature was kept above 50°C. The resultant slurry was stirred at 55°C for 1h, cooled to room temperature and stirred overnight. The slurry was filtered and washed with water (3x25ml). The residue was added to IMS (50ml) and the slurry was heated to 45°C and stirred overnight. The slurry was cooled to room temperature, filtered and the damp cake was dried *in vacuo* at 55°C to the title compound as a white solid (3.40g).

Chiral chromatographic analysis showed that the required product of the reaction contained 0.7% of the unwanted (-) enantiomer.

Pharmacy Examples

Oral solution

Active ingredient	0.5-800 mg
Polyethylene glycol 400 NF	q.s. to 50 ml

The active ingredient is suspended in Polyethylene glycol 400 and is then dissolved by sonication to produce the oral solution.

Oral suspension

Active ingredient	0.5-80 mg
Polysorbate 80 NF (Tween 80)	0.02 ml
Sterile Water for Irrigation	q.s. to 20ml

The active ingredient is added to a 0.1% (v/v) Tween 80 solution (20 ml) and the mixture is then sonicated or shaken to produce the oral suspension.

Tablets

a.

Active ingredient	6mg
Lactose anhydrous USP	136.2mg
Sodium Starch Glycolate USP/NF	6mg
Stearic Acid USP/NF	1.5mg
Colloidal Silicon Dioxide USP/NF	0.3mg

	Compression weight	150mg

The active ingredient, lactose, and sodium starch glycolate are sieved through a 590 micron sieve and blended in a suitable mixer. Stearic acid (screened through a 250 micron sieve) and colloidal silicon dioxide are added to and blended with the active blend. The blend is compressed into tablets using suitable punches.

b.	Active ingredient	6mg
	Microcrystalline cellulose USP/NF	136.5mg
	Crospovidone USP/NF	6mg
	Magnesium stearate USP/NF	1.5mg
	Compression weight	150mg

The active ingredient, microcrystallline cellulose and crospovidone are sieved through a 590 micron sieve and blended in a suitable blender. The magnesium stearate is screened (through a 250 micron sieve) and blended with the active blend. The resultant blend is compressed into tablets using suitable tablet punches.

Capsules

a.	Active ingredient	6mg
	Microcrystalline cellulose USP/NF	128.25mg
	Sodium starch glycolate USP/NF	15mg
	Magnesium stearate USP	0.75mg
	Fill weight	150mg

The active ingredient, microcrystalline cellulose, and sodium starch glycolate are screened through a 590 micron mesh sieve, blended together and lubricated with magnesium stearate, that has been screened through a 250 micron sieve. The blend is filled into capsules of a suitable size.

b.	Active ingredient	6mg
	Lactose monohydrate USP	130.5mg
	Povidone USP	6mg

Crospovidone NF	6mg
Magnesium stearate	1.5mg
Fill weight	150mg

The active ingredient and lactose are blended together and granulated with a solution of Povidone. The wet mass is dried and milled. The magnesium stearate and Crospovidone are screened through a 250 micron sieve and blended with the granule. The resultant blend is filled into hard gelatin capsules of a suitable size.

BIOLOGICAL ASSAYS

The (+) and (-)-enantiomers and the racemic mixture were characterized in the following assays. The results of these assays are summarized in the table below.

Guinea Pig Gallbladder Tissue Preparation. Gallbladders were removed from male Hartley guinea pigs sacrificed with CO_2 atmosphere. The isolated gallbladders were cleaned of adherent connective tissue and cut into two rings from each animal (2-4 mm in length). The rings were suspended in organ chambers containing a physiological salt solution (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_3, 25 mM $NaHCO_3$, 11.1 mM dextrose). The bathing solution was maintained at 37°C and aerated with 95% O_2/5% CO_2 to maintain pH = 7.4. Tissues were connected via gold chains and stainless steel mounting wires to isometric force displacement transducers (Grass, Model FT03 D). Responses were then recorded on a polygraph (Grass, Model 7E). One tissue from each animal served as a time/solvent control and did not receive test compound. Rings were gradually stretched (over a 120-min. period) to a basal resting tension of 1 gm which was maintained throughout the experiment. During the basal tension adjustment period, the rings were exposed to acetylcholine (10^{-6} M) four times to verify tissue contractility. The tissues were then exposed to a submaximal dose of sulfated CCK-8 (Sigma, 3 X 10^{-9} M).

After obtaining a stable response, the tissues were washed out 3 times rapidly and every 5 to 10 minutes for 1 hour to reestablish a stable baseline.

Agonist EC50's. Compounds were dissolved in dimethylsulfoxide (DMSO) then diluted with water and assayed via a cumulative concentration-response curve to test compound (10^{-11} to 3 X 10^{-5} M) followed by a concentration-response curve to sulfated CCK-8 (10^{-10} to 10^{-6} M) in the presence of the highest concentration of the test compound. As a final test, acetylcholine (1 mM) was added to induce maximal contraction. A minimum of three determinations of activity were made for each test compound.

Establishment of stable CCK receptor bearing cell lines. The cDNA clones for the human CCK-A[18] or CCK-B[19] receptors were ligated into pcDNA1-Neo vector from Invitrogen Corp (San Diego, CA) for direct transfection. DNA was prepared by the alkaline lysis method and transfected into CHO-K1 cells (ATCC, Rockville, MD) using the Lipofectin reagent[24] (Gibco BRL, Gaithersberg, MD). Stable transfectants were initially selected by the use of Geneticin (Gibco BRL) and receptor bearing resistant cells were enriched by fluorescence-activated cell sorting based on binding of Fluorescein-Gly-[(Nle28,31]-CCK-8. Clonal lines were subsequently established by the limiting dilution method.

Cell Membrane Preparation. CHO-K1 cells stably transfected with human CCK-A or CCK-B receptor cDNA were grown at 37°C under a humidified atmosphere (95% O_2/5% CO_2) in Ham's F12 medium supplemented with 5% heat inactivated fetal bovine serum. The cells were passaged twice weekly and grown to a density of 2-4 million cells/mL. The cells were collected by centrifugation (600 X g, 15 min, 4°C) and resuspended in buffer (20 mL, pH 7.4) containing TrisHCl (25 mM), EDTA (5 mM), EGTA (5 mM), phenyl sulfonyl fluoride (0.1 mM) and soybean trypsin inhibitor (100 μg/mL). Cells were disrupted with a motorized glass teflon homogenizer (25 strokes) and the

homogenate was centrifuged at low speed (600 X g, 10 min, 4°C). The supernatent was collected and centrifuged at high speed (500,000 X g, 15 min 4°C) to pellet the particulate fraction. The low speed pellet was processed three additional times. High-speed particulate fractions were combined and resuspended in buffer (1-5 mg protein/mL) and frozen at -80°C. Protein concentration was determined according to manufacturer's directions using BioRad reagent and bovine serum albumin as standard.

Receptor Binding Assays. ^{125}I-Bolton Hunter CCK-8 (Amersham, 2000 Ci/mmol) was dissolved in binding buffer (pH 7.4, 100,000 cpm/25 µL) containing HEPES (20 mM), NaCl (118 mM), KCl (5 mM), MgCl2 (5 mM) and EGTA (1 mM). Nonspecific binding was determined with MK-329[20] (10 µM, CCK-A) or L-365,260[21] (10 µM, CCK-B). Test compounds were dissolved in DMSO at a stock concentration of 100 times the final assay concentration and diluted to appropriate concentrations with binding buffer. Binding assays were performed in triplicate using 96-well plates to which the following were added sequentially: test compound (25 µL), ^{125}I-Bolton Hunter CCK-8 (25 µL), buffer (pH 7.4, 150 µL) and receptor preparation (50 µL). The final concentration of DMSO was 1% in all assay wells. After 3 hours at 30°C, the incubation was terminated by rapid filtration of the mixture onto glass filters (Whatman GF/B) with subsequent washing to remove unbound ligand. Bound radioactivity was quantified by gamma counting.

Intracellular Calcium Measurements: CHO-K1 cells stably transfected with hCCK-A or hCCK-B receptors were grown on glass coverslips to 75-90% confluency. The cells were loaded for 50 minutes in serum-free culture medium containing 5 mM FURA2-AM and 2.5 mM probenecid. A JASCO CAF-102 calcium analyzer was used to measure changes in intracellular calcium concentration by standard ratiometric techniques using excitation wavelengths of

340 nm and 380 nm. Cells were perfused with increasing concentrations of CCK-8 (n = 2) or compounds (n = 2) until a plateau in the 340/380 ratio was achieved. A washout/recovery period of at least 10 minutes was allowed between successive stimulations. The maximal response was normalized to the maximal response induced by CCK-8. EC_{50}'s were calculated at the concentration required to induce half-maximal response. In addition to the agonist concentration-response curves, the CHO-K1 cells expressing the human CCK-B receptor were perfused for 1 hour with three concentrations of compounds (10^{-8}, 10^{-7}, 10^{-6} M, n = 2), then a concentration response curves were acquired for CCK-8 (10^{-12} to 10^{-6}M).

Anorexia Assays: Male Long-Evans rats (225-300 g) were conditioned for two weeks to consume a palatable liquid diet (Bio-Serve F1657, Frenchtown, NJ) after a 2 hr fast. On pretreatment day, rats were fasted (100 min) and injected IP with drug vehicle (propylene glycol, PG, 1 mL/kg) and an oral preload of saline (0.9% NaCl, 8 mL/kg). Liquid diet access was provided 20 min later and consumption was measured at 30, 90 and 180 min. To qualify for the drug treatment study, rats had to consume at least 8 mL of liquid diet within the first 30 minutes on the pretreatment day. The next day, following the 100 min deprivation, rats (8 - 10 animals per dose) were treated IP or PO with vehicle (PG, 1 mL/kg) or various doses (0.01 to 10 μmol/kg) of test compound dissolved in PG (1 mL/kg), immediately followed by the saline oral preload. Food access was again provided 20 min later and food intake was measured at 30, 90 and 180 min. All food intake data were normalized for each rat to the respective values from the pretreatment day. Potency was determined at 30 min and efficacy at the 30 min, 1 μmol/kg dose.

Mouse gallbladder emptying assay: Makovec, F.; Bani, M.; Cereda, R.; Chiste, R.; Pacini, M. A.; Revel, L.; Rovati, L. C. Antispasmodic Activity on the Gallbladder of the Mouse of CR1409 (Lorglumide), a Potent Antagonist of Peripheral Chlolecystokinin. *Pharmacol. Res. Commun.* **1987**, *19*, 41-51.

PApp values: A measurement of intestinal permeation determined using the *in vitro* assay of Artursson P. and Karlson J. 1991, Biochem. Biophys Res. Common.175, 880-885 (Correlation between oral drug absorption in humans and apparent drug permeability co-efficients in human intestinal epithelical (CACO-2) cells).

Pharmacological Comparison of Enantiomers & Racemate

Assay	(+) Enantiomer	(-) Enantiomer	Racemate
In vitro			
GPGB EC_{50} (nM)	**9.3**	63	40
HCCK-A IC_{50} (nM)	148	191	123
HCCK-A EC_{50} (nM)	150	298	252
HCCK-B K_i (nM)	3.2	22.3	10
HCCK-B EC_{50}(nM)	Antagonist	Antagonist	Antagonist
Selectivity Ratio[1]	**46**	8.6	12.3
PApp (x 10^{-7} cm/sec)	1.6		0.7
in vivo			
Rat Anorexia			
ED_{50} IP (μmol/kg)	0.034	0.48	0.06
ED_{50} PO (μmol/kg)	1.1	Inactive[3]	2.0
Mouse Gallbladder			
Emptying Assay			
ED_{50} IP (μmol/kg).	0.002	0.012	0.007
ED_{50} PO (μmol/kg)	**0.007**	Inactive[2]	0.055

1. Selectivity ratio = IC50 (hCCK-A)/Ki (hCCK-B)
2. Inactive up to 10 μmol/kg
3. No significant reduction in feeding observed with doses up to 10μmol/kg.

Three unexpected biological activities distinguish the (+) enantiomer from the (-) enantiomer and the racemate. Two of these activities relate to enhanced CCK-A efficacy, which should improve the beneficial activity of this enantiomer. The third relates to the CCK-B antagonist activity of the (+) enantiomer which should prove beneficial through decreased toxicity.

The (+) enantiomer was four-fold more potent than the racemate in the in vitro isolated guinea pig gallbladder test ("GPGB"). The (+) enantiomer was eight-fold more potent than the racemate in the mouse gallbladder emptying assay (oral dosing). This increased potency is expected to be beneficial in the treatment of gallbladder stasis and in the treatment of obesity, since gallbladder stasis is a critical problem with rapid weight loss.

Anorectic agents are intended for chronic use and thus it is essential that they possess minimal risk for toxicity. The primary toxicity associated with the use of cholecystokinin is concomitant CCK-B receptor agonist activity. Activation of the CCK-B receptor is primarily associated with increased anxiety and increased gastric acid secretion. The utility of CCK-B antagonists have been explored for both the development of anxiolytic agents and anti-ulcer agents. See, for example, Lowe, J, "Cholecystokinin-B Receptor Antagonists" in Exp. Opin. Ther. Patents, 5(3), pp 231-237 (1995).

The predominant CCK receptor subtype in the rodent pancreas is the CCK-A subtype and activation of this subtype induces pancreatic hyperstimulation and hypertrophy in rodents. Both of these activities are considered to be undesirable. Recently, the tissue distribution of CCK receptors in human tissues has been reported. Surprisingly, the predominate receptor subtype in human pancreas is the CCK-B receptor subtype. See, for example, Wank, S. A., "Cholecystokinin Receptors" in American Journal of Physiology - Gastrointestinal & Liver Physiology, 32(5):, pp G628-G646, (1995). Thus, in humans, activation of the CCK-B receptor (CCK-B agonist activity) could induce increased anxiety and

gastric acid secretion, as well as pancreatic hyperstimulation and hypertrophy with long term use.

In order to decrease the risk of undesirable in vivo CCK-B agonist activity, the preferred compound should have affinity for the CCK-B receptor and have measurable CCK-B antagonist activity in in vitro assays. Both enantiomers and the racemate are CCK-B antagonists. Although all three compositions have similar human CCK-A receptor affinities (IC50) and efficacies (EC50), the (+) enantiomer has the highest CCK-B receptor affinity (IC50) and selectivity (46-fold). Thus, the (+) enantiomer is preferred both in terms of CCK-A potency and efficacy, as well as in terms of the minimal potential for CCK-B induced toxic side effects.

The (+) enantiomer of the invention is essentially non-toxic at therapeutically useful doses. For example, in single dose oral studies the maximum non-lethal dose was found to be greater than 2000mg/kg in the rat and 1000mg/kg for male mice and 500mg/kg for female mice.